

Addition of Cd²⁺ Metal Ions to Conway Culture Medium on Phytoplankton Growth of *Chaetoceros calcitrans*

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Abstract— The main objective of this study is to examine the impact of Cd²⁺ metal ions on the growth of marine phytoplankton, specifically focusing on their potential as agents for phytoremediation in marine settings affected by heavy metal pollution. In this study, the behavior of Cd²⁺ metal ions added to the culture medium in phytoplankton type of *Chaetoceros Calcitrans* in the culture medium, an investigation was conducted on a series of *Chaetoceros Calcitrans* cultures, comparing those including and not including the Cd²⁺ metal ions' addition. Observations were made on the growth pattern of *Chaetoceros Calcitrans*. To assess the impact of introducing Cd²⁺ metal ions into the Conway culture media, various metrics such as definite growth rate, growth inhibition percentage, and test of toxicity were employed. The findings indicated that the growth trajectory of *Chaetoceros Calcitrans* in the Conway medium, in the absence of Cd²⁺ metal ions as a control group, exhibited the most substantial growth curve. The growth patterns observed in the culture medium upon the addition of Cd²⁺ metal ions at a concentration of 0.1 mg/L were found to be comparable to those observed in the samples of control group. Adding Cd²⁺ metal ions at concentrations exceeding 0.1 mg/L has decreased the inhibited growth rate of *Chaetoceros Calcitrans*. The concurrent increase in PGI costs further exacerbates this effect. The findings from the statistical analysis of difference tests conducted on blanks investigating the impact of introducing Cd²⁺ metal ions to *Chaetoceros Calcitrans* suggest that concentrations ranging from 0.01 to 0.10 ppm of Cd²⁺ metal ions have no discernible effect on the growth of *Chaetoceros Calcitrans*. Furthermore, the highest concentration of Cd²⁺ metal ions that *Chaetoceros Calcitrans* can withstand is 0.10 ppm, with an EC₅₀ value of 6.13 ppm.

Keywords—*Chaetoceros Calcitrans*; Cd²⁺ metal ion; inhibited growth rate.

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I. INTRODUCTION

Phytoplankton, which are diminutive, photosynthetic creatures, inhabit many aqueous habitats such as oceans, seas, lakes, and rivers. Phytoplankton are of utmost importance in the Earth's ecology [1]–[3], as they fulfill a vital role as primary producers within aquatic food chains [4]–[7]. They serve as a major source of sustenance for a diverse range of marine and aquatic animals. Like terrestrial plants, phytoplankton employs the process of photosynthesis to transform sunlight, carbon dioxide, and essential nutrients (e.g., nitrogen and phosphorus) into organic substances and oxygen. The process is crucial in producing atmospheric oxygen and carbon dioxide sequestration from marine environments.

Phytoplankton has diverse species, encompassing numerous forms of algae such as diatoms, dinoflagellates, green algae, and cyanobacteria, commonly known as blue-green algae [8]–[10]. Organisms of many morphologies and

dimensions are observed, ranging from minuscule unicellular entities to more substantial colonial structures. Phytoplankton occupies the foundational position within aquatic food webs [4], [11], [12]. Zooplankton, reduced-sized piscine species, and additional aquatic organisms derive sustenance from the aforementioned organisms.

Primary production plays a crucial role in supporting the existence of various marine organisms, such as whales, dolphins, and a diverse range of fish species [13]–[15]. Under inhibited circumstances, phytoplankton populations have the potential to undergo rapid proliferation, commonly referred to as "bloom" episodes [16]–[18]. These blooms can be attributed to various circumstances, such as increased nutrient levels, elevated water temperatures, or other contributing elements. Blooms play a crucial role in sustaining marine life [16], [17], [19]; however, an overabundance of blooms can occasionally result in harmful algal blooms (HABs). These HABs have the potential to discharge toxins that pose a threat to both marine organisms and human beings [20]–[23].

Phytoplankton plays a crucial role in the global carbon cycle [24]–[27]. During photosynthesis, they actively participate in the absorption of substantial quantities of carbon dioxide, playing a crucial role in mitigating the presence of this greenhouse gas inside the Earth's atmosphere. Upon their demise and subsequent descent to the oceanic abyss, these organisms possess the ability to effectively sequester carbon over extended durations.

Phytoplankton populations are subject to alterations that can function as reliable markers of environmental well-being [28]–[30]. Various variables, including climate change, pollution, and the discharge of nutrients from agricultural and urban regions drive these changes. Phytoplankton is frequently examined by scientists as a means of monitoring the condition of aquatic ecosystems [31], [32]. Certain species of phytoplankton exhibit bioluminescence, a phenomenon characterized by their ability to generate light. This naturally occurring phenomenon can produce captivating exhibitions of luminescent water, particularly during nighttime. Phytoplankton plays a crucial role in maintaining the ecological balance of aquatic systems and significantly influences global climate patterns and the overall welfare of various organisms, including humans. Marine organisms play a vital role in the marine food web and contribute significantly to regulating carbon and oxygen cycles on a global scale [31], [33].

Phytoplankton is a complex assemblage of microscopic plant creatures, each exhibiting unique traits. Phytoplankton has a crucial role as a primary producer within the aquatic food chain, serving as a fundamental source of nutrition for zooplankton [34], [35]. Consequently, the zooplankton species are subsequently exposed to predation by larger organisms, ultimately resulting in their eating by humans. On the contrary, water is known to contain heavy metal ions as dissolved particles [36]–[39]. The efficiency of phytoplankton-heavy metal ions interaction in aquatic environments is attributed to the comparatively tiny size of phytoplankton.

Therefore, one could contend that phytoplankton assumes a crucial function in disseminating biological substances, acting as the principal conduits via which pollutants infiltrate the food web and constituting the most significant biomass within aquatic ecosystems. The uptake of heavy metal ions by phytoplankton results in a substantial enhancement of their efficacy. Moreover, phytoplankton exhibits the capacity to flourish in aqueous habitats that are polluted with heavy metal ions [36]–[38], [40]. This proposition recommends that researchers investigate the possible application of phytoplankton as a phytoremediation strategy for addressing heavy metal pollution.

Cadmium is an elementary substance with the chemical symbol “Cd” and atomic number 48 [34], [41], [42]. This specific chemical element is distinguished by its inherent rarity and is frequently found in restricted quantities inside the lithosphere of the Earth. Cadmium is acknowledged for its distinctive bluish-white coloration [43]–[45]. Due to its remarkable characteristics, it is commonly utilized in many industrial settings. Cadmium exhibits distinctive properties, such as its inherent softness, malleability, and [46]–[48]. The material demonstrates a relatively elevated density, approximately 8.65 grams per cubic centimeter [49].

Cadmium is not naturally occurring in its elemental state but is commonly found in conjunction with zinc ores, such as sphalerite (zinc sulfide), and several other minerals [50]. It is frequently acquired as a secondary product during zinc refining. Historically, cadmium has been employed in rechargeable nickel-cadmium (NiCd) batteries owing to its exceptional electrical conductivity and effective electrical energy storage capabilities [51]. Nevertheless, the utilization of this substance in batteries has experienced a decrease in popularity, as it has been superseded by alternative possibilities that are more ecologically sustainable.

Cadmium compounds find application in manufacturing pigments utilized in creating ceramics, plastics, and paints, such as the production of color [52], [53]. The pigments in question are renowned for their vivid and enduring hues. Cadmium is employed in electroplating to impart corrosion resistance to steel and other materials [54]. Cadmium is employed as a neutron absorber in certain nuclear reactors to fulfill control and safety functions [55]. Historically, cadmium-based solder has been used in some applications; however, its usage has declined due to growing apprehensions regarding its environmental impact. Cadmium is toxic to both humans and the surrounding ecosystem [56]. The potential health consequences of cadmium exposure, whether inhalation or ingestion, are significant and encompass conditions, such as lung and prostate cancer, renal impairment, and various other health complications.

As a result of these issues, stringent rules exist about the utilization and proper disposal of the substance above. Numerous nations have implemented regulations on the permissible cadmium levels in consumer goods such as toys, jewelry, and electronics. Various initiatives have been undertaken to mitigate the environmental consequences of using cadmium in diverse applications. Numerous endeavors have been undertaken to identify alternative materials to replace cadmium, primarily driven by its inherent toxicity. In contemporary consumer devices, nickel-cadmium batteries have been mainly supplanted by nickel-metal hydride (NiMH) and lithium-ion batteries [57]. According to safety protocols, it is imperative to exercise caution when dealing with cadmium and its derivative products to avert exposure and mitigate potential adverse effects on human well-being and the natural environment.

Cadmium (Cd) possesses a relative atomic mass of 112.40 grams per mole, as indicated by its placement in the Periodic Table. It is classified within group IIB, alongside zinc (Zn) and mercury (Hg) [58]. In natural environments, the prevailing condition is the presence of the metal ion Cd^{2+} , which tends to form compounds with other metal ions such as Zn^{2+} according to carbonate and sulfate minerals. Within the context of the Pb and Zn metal mining sector, the purification procedure consistently yields by-products comprising cadmium metal ions, which are then discharged into the environment as waste. Cadmium has found extensive application across several industries, encompassing metal plating, smelting, dyeing, batteries, plastics, printing, textiles, lubricating oils, and fuels [59]. The study conducted by Moore and Ramamoorthy [60] demonstrated that cadmium metal ions exhibit covalent binding properties, possess a strong affinity for thiol groups, enhance fat solubility, and have the ability to accumulate, resulting in hazardous effects.

The Cd²⁺ metal ions have the ability to induce oxidative stress, hence inhibiting the synthesis of reactive oxygen species (ROS) [61]. Additionally, in the presence of H₂O₂, these metal ions can generate free radicals in vitro [62]. In an in vitro setting, the presence of elevated concentrations of Cd²⁺ metal ions possesses the capacity to induce deleterious effects on the reverse reaction of proteins [62] by forming metal-thiolate linkages [63] and altering the permeability of cell walls or membranes through attaching to nucleophile groups [64].

The aforementioned influence has the potential to directly or indirectly induce the formation of reactive oxygen species (ROS), thereby disrupting redox processes and influencing the decrease in chlorophyll synthesis [65], [66]. The presence of reactive oxygen species (ROS) is associated with a high level of toxicity, which has cytotoxic effects. ROS can interact with lipids, proteins, and nucleic acids, causing lipid peroxidation and dysfunction of membranes and enzymes. The primary objective of this study is to investigate the influence of Cd²⁺ metal ions on the growth of marine phytoplankton, with a particular emphasis on their potential as agents for phytoremediation, designed for heavy metal pollution in marine environments. Observed growth parameters included the inhibited growth rate, the growth inhibition percentage, and the toxicity test.

II. MATERIALS AND METHOD

A. Materials

The materials utilized in this study encompass the following:

- *Tetraselmis chui* phytoplankton seedlings from pure culture of the Maros Fisheries and Marine Research Institute, South Sulawesi.
- All the substances utilized in the experiment are of analytical grade, which include CdCl₂.2, 5H₂O, K₂Cr₂O₇, CuCl₂.2H₂O, EDTA(C₁₀H₁₆N₂O₈), GSH, SiO₂, (C₁₀H₁₇N₃O₆S), FeCl₃.6H₂O, MnCl₂.4H₂O, H₃BO₃, NaH₂PO₄.2H₂O, NaNO₃, Aquabidest, ZnCl₂, CoCl₂.6H₂O, (NH₄)₆MoO₄.4H₂O, CuSO₄.5H₂O, Vitamin B₁₂, Vitamin B₁, Na₂SiO₃.5H₂O. Buffer Solution, Whatman filter paper 42.

The equipment used is distinguished by:

- Glassware consists of culture containers, including 500 mL bottles, 60-liter aquariums, and a set of glassware.
- Measuring instruments, including pH meter, salinometer, Ohaus digital balance NO AP 110, microscope, SPNISOSFD oven, FT-IR Shimadzu, and Atomic Absorption Spectrophotometry (AAS).
- Additional tools utilized in this study encompass a vacuum pump, porcelain die, aerator, refrigerator, plastic hose, pan, electric burner, neon balloon, thermometer, panel filter with a diameter of 47 mm, magnetic stirrer, hand counter, hemocytometer, Air Condition unit, and a 25 mL film container.

B. Research Procedure

The procedure of this research is divided into three steps. The first step is investigating the growth pattern of *Chaetoceros Calcitrans* Phytoplankton in the Conway Culture Medium. The second step is to examine the effect of Cd²⁺

metal ions on inhibited growth rate and percentage of phytoplankton growth resistance added to Cd²⁺ metal ions. The last is tested to toxicity of Cd²⁺ metal ions on phytoplankton. The research procedures are shown in Fig. 1.

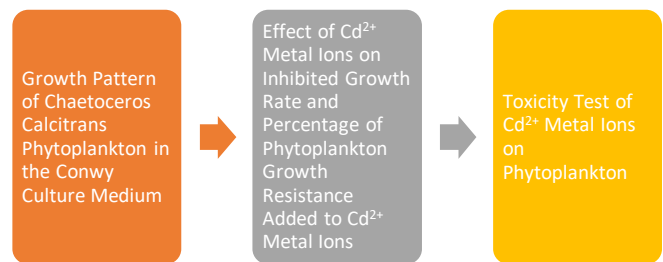


Fig. 1 Research procedures

1) Growth Pattern of *Chaetoceros Calcitrans* Phytoplankton in the Conway Culture Medium:

Chaetoceros Calcitrans phytoplankton was cultivated using a 500 mL Erlenmeyer flask containing sterile seawater media. Before the experiment, the saltwater medium was filtered, and its salinity and pH were evaluated. Subsequently, 2 mL of Conway solution, 2 mL of vitamin solution, and 2 mL of silicate solution per liter were added to the medium. During the process of culture implementation, it is necessary to maintain many physic-chemical parameters. These parameters include the continuous provision of illumination using a 40-watt fluorescent lamp, the introduction of CO₂ gas using an air pump aerator, and maintaining a temperature range of 25-27°C; the objective is to sustain a pH level within the range of 8-9 in the medium, while also guaranteeing a salinity level of 30‰. Before usage, all materials utilized in the culturing process are subjected to sterilization.

Following the implementation of an 8-day culture period, the phytoplankton seedlings undergo subsequent cultivation in culture bottles with a capacity of 1 liter. To ascertain the growth pattern of phytoplankton, a daily assessment is conducted to calculate the number of cells per milliliter of the medium. Phytoplankton-grown media samples were collected using sterile droppers; approximately 0.5 mL of the samples were placed on a Hemocytometer and subsequently examined under a microscope [67]. Given that the cell density remains within the expected range, one can proceed to calculate the density utilizing the designated formula:

$$Sum = \frac{\text{Total number of cells in 4 squares}}{\text{Number of blocks}} \times 10,000 \quad (1)$$

2) Effect of Cd²⁺ Metal Ions on Inhibited Growth Rate and Percentage of Phytoplankton Growth Resistance Added to Cd²⁺ Metal Ions:

The present study aims to investigate the impact of Cd²⁺ metal ions on the growth of *Chaetoceros Calcitrans*. To achieve this, phytoplankton were cultured in a medium supplemented with a solution containing Cd²⁺ metal ions at concentrations ranging from 0 to 5 ppm. Equation (2) is employed to calculate the inhibited growth rate for each concentration change, whereas equation (3) is utilized to determine the percentage of growth inhibition (PGI) in phytoplankton.

$$\mu = \frac{\ln N_t - \ln N_0}{t} \quad (2)$$

$$PGI = 100 - \left(\frac{\mu_i}{\mu_0} \times 100\% \right) \quad (3)$$

3) Toxicity Test of Cd²⁺ Metal Ions on Phytoplankton:

The study entailed evaluating the influence of Cd²⁺ metal ions on the proliferation of phytoplankton via a toxicity assay. The phytoplankton were cultured under ideal conditions, utilizing a culture capacity of 1000 mL. The parameters examined in this study encompass the Non-Effect Concentration (NEC) test aims to ascertain Cd²⁺ metal ions' concentration that does not exert any discernible impact on phytoplankton growth. The Maximum Tolerable Concentration (MTC) was also determined to establish the highest concentration of Cd²⁺ metal ions that phytoplankton can withstand with no adverse effects. Finally, the EC50 (Effect Concentration 50%) was established to ascertain the concentration of Cd²⁺ metal ions that results in a 50% decrease in the growth rate of phytoplankton compared to control samples.

The cell density (μ) was obtained by quantifying the concentration of Cd²⁺ metal ions that were introduced. Following this, a distinct examination was carried out on samples devoid of identifiable information, employing a confidence level of 99%. This allowed for a price calculation; the t-table value was compared to each variation in the concentration of Cd²⁺ metal ions. If the calculated value of t-table is less than the value of the t-count, the NEC value is determined by arranging the prices in ascending order. The MTC value is determined by selecting the highest price from the calculated value of t-table. The EC₅₀ value is determined

by plotting the PGI price against the concentration of Cd²⁺ metal ions added.

III. RESULTS AND DISCUSSION

A. Growth Patterns of Phytoplankton *Chaetoceros Calcitrans*

The growth pattern of marine phytoplankton *Chaetoceros Calcitrans* was observed in a seawater medium supplemented with Conway medium. The experiment was conducted under controlled physico-chemical conditions, which included continuous exposure to lighting provided by two 40-watt TL lamps placed approximately 75 cm away from the culture. The medium was aerated with CO₂ gas from an air pump, and the temperature was maintained between 25-27°C. The pH of the medium was regulated to be within the range of 8-9, and the salinity of the medium was set at 30‰. The phytoplankton species *Chaetoceros Calcitrans* typically undergoes four distinct stages of growth. *Chaetoceros calcitrans* exhibits a rapid adaptation period of around two days to acclimate to its growing medium, facilitating optimal cell division between days two and six. The utilization of Conway media for the cultivation of marine phytoplankton *Chaetoceros Calcitrans* at an initial density of 250,000 cells/mL of the medium has been seen to result in a substantial rise in density, reaching around 29 times its starting value within a span of 6 days of cultivation. The inhibited growth rate constant (μ) of phytoplankton *Chaetoceros Calcitrans* cultivated in Conway medium is determined by calculating the cell density of every milliliter of a medium by equation (2). The resulting values are reported in Table 1.

TABLE I
CELL DENSITY AND INHIBITED GROWTH RATE CONSTANT (μ) *CHAETOCEROS CALCITRANS* CULTURED USING CONWAY MEDIUM

| Density/ μ <i>Chaetoceros Calcitrans</i> | Days | | | | | | | | | |
|---|------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| Density ($\times 10^4$ cell mL ⁻¹) | 25 | 40 | 110 | 276 | 448 | 618 | 740 | 872 | 0.984 | 1.271 |
| μ (day ⁻¹) | - | 0.470 | 0.741 | 0.801 | 0.721 | 0.642 | 0.565 | 0.507 | 0.459 | 0.436 |

According to the data presented in Table 1, the cultivation of *Chaetoceros Calcitrans* seedlings in Conway medium exhibits a favorable growth trend. Specifically, the cell density demonstrates an upward trend as the number of culture days increases until day 9. This can be attributed to the provision of limited nutrients, which restricts the period of cell growth. However, on day ten, there is a decline in cell count within the medium. Based on the observed inhibited growth rate of *Chaetoceros Calcitrans*, it can be inferred that the culture experiences a strong growth phase on the third day, followed by a progressive drop-in growth rate.

Table 1 presents a steady increase in the inhibited growth rate of the phytoplankton species *Chaetoceros Calcitrans*. Therefore, the rapid attainment of optimal development for phytoplankton is observed. Hence, utilizing the inhibited growth rate constant parameter enables the estimation of the duration (in days) required for a culture to attain its optimal density.

B. Inhibited Growth Rate and Percentage of Growth Retardation of *Chaetoceros Calcitrans* in Medium Added to Cd²⁺ Metal Ions

Figure 2 illustrates the growth trend of *Chaetoceros Calcitrans* in the presence of Cd²⁺ metal ions ranging between 0.10 and 10.00 ppm, specifically added by cadmium chloride, within a culture medium for marine phytoplankton. According to the data presented in Figure 2, the development pattern of *Chaetoceros Calcitrans* in Conway medium without the inclusion of Cd²⁺ metal ions (referred to as the control group) exhibits the most pronounced growth curve. The growth patterns seen in the culture medium with the addition of Cd²⁺ metal ions at a concentration of 0.10 milligrams per liter (mg/L) were found then to compare to the control group.

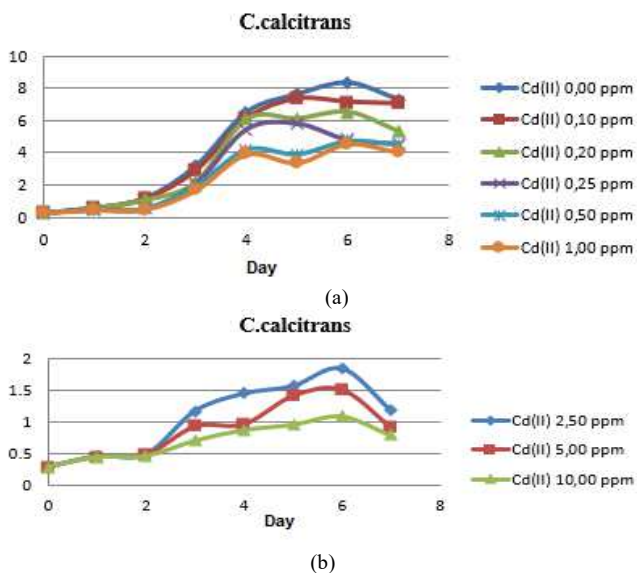


Fig. 2 Growth pattern of *Chaetoceros Calcitrans* under conditions including and not including the addition of Cd²⁺ metal ions at various levels of concentration

The growth pattern graph exhibits a negative correlation with the concentration of Cd²⁺ metal ions, indicating that the growth pattern decreases as the concentration of Cd²⁺ metal ions increase. The observation provides evidence that Cd²⁺ metal ions' addition in the culture medium of *Chaetoceros*

Calcitrans could result in a reduction in the growth of phytoplankton, as depicted in Table 2. This observation aligns with the findings proposed by Foster [68], indicating that the impact of heavy metals on unicellular plankton is typically linked to a reduction in both cell abundance and biomass.

According to the findings presented in Table 2, it can be observed that, across all treatments, including differences in the concentration of Cd²⁺ metal ions, there is a general trend of an initial increase in the inhibited growth rate as the culture time progresses. However, this increase is followed by a partial drop in the inhibited growth rate after day 3. The reason for this phenomenon can be attributed to an ample supply of nutrients within the medium, which facilitates phytoplankton growth. However, the detrimental impact of Cd²⁺ metal ions reduce the inhibited growth rate of *Chaetoceros Calcitrans*. On the first day, the experiment observed the effects of a Cd²⁺ metal ion concentration of 0.50 ppm on the growth of phytoplankton. This concentration resulted in the lowest growth rate ($\mu = 0.504 \text{ day}^{-1}$) and a decrease in the photosynthetic growth index (PGI) to 34.1%. Even after seven days, adding 0.5 ppm Cd²⁺ metal ions to the medium negatively impacted cell growth, with a reduced growth rate of $\mu = 0.397 \text{ day}^{-1}$. Consequently, it exhibits a gradual augmentation in the inhibited rate. The addition of Cd²⁺ metal ions lead to a reduction in the phytoplankton-inhibited growth rate constant.

TABLE II
INHIBITED GROWTH RATE CONSTANT (μ) AND PGI *CHAETOCEROS CALCITRANS* INCLUDING AND NOT INCLUDING CD²⁺ METAL IONS' ADDITION AT VARIOUS LEVELS OF CONCENTRATION

| [Cd ²⁺] (ppm) | $\mu \text{ (day}^{-1}\text{) and PGI (\%)\ day}$ | | | | | | |
|---------------------------|---|--------|--------|--------|--------|--------|--------|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| 0.00 | 0.764 | 0.730 | 0.814 | 0.789 | 0.662 | 0.567 | 0.467 |
| | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 0.10 | 0.754 | 0.718 | 0.782 | 0.773 | 0.654 | 0.541 | 0.462 |
| | 1.322 | 1.678 | 3.923 | 2.028 | 1.225 | 4.614 | 1.091 |
| 0.20 | 0.744 | 0.681 | 0.689 | 0.772 | 0.618 | 0.526 | 0.422 |
| | 2.657 | 6.719 | 15.367 | 2.158 | 6.631 | 7.200 | 9.627 |
| 0.25 | 0.542 | 0.319 | 0.672 | 0.745 | 0.608 | 0.475 | 0.400 |
| | 29.086 | 56.347 | 17.471 | 5.568 | 8.148 | 16.261 | 14.305 |
| 0.50 | 0.504 | 0.304 | 0.646 | 0.677 | 0.527 | 0.471 | 0.397 |
| | 34.109 | 58.327 | 20.612 | 14.196 | 20.367 | 16.912 | 14.983 |

The empirical evidence demonstrates that there is an addition of hazardous metal ions, specifically Cd²⁺. The culture medium cannot be counteracted by an adequate supply of nutrients, as indicated by the data on inhibited growth rates and growth inhibitions percentage. According to the findings of Wang and Evangelou [69], it has been observed that the composition of phytoplankton cell walls typically consists of around 25-30% cellulose, 15-25% hemicellulose, 35% pectin, and 5-10% glycoprotein. Cellulose, pectin, glycoproteins, and certain enzymes containing Zn possess functional groups such as carboxylates, thiols, and C=O and S-H groups. These functional groups have the ability to engage in interaction with Cd²⁺ metal ions through covalent bonds or ion exchange. The soft base nature of the C=O and S-H groups facilitates strong binding with Cd²⁺ metal ions. Additionally, within each cell, approximately 260 types of enzymes rely on Zn²⁺ metal ions for proper functioning. Cd²⁺ metal ions can replace Zn²⁺ metal ions, leading to enzyme dysfunction and disruption of phytoplankton cell tissue [70].

C. Cd²⁺ Metal Ion Toxicity Test against *Chaetoceros Calcitrans*

The present study presents the results of a statistical analysis conducted on blank samples to investigate the impact of introducing Cd²⁺ metal ions to *Chaetoceros Calcitrans* at various doses ranging from 0.1 to 10.00 ppm. The findings additionally demonstrated the computed values of 2.443, 13.951, 33.905, 54.885, 67.291, 199.142, 211.099, and 383.345. The critical value for a significance level of $\alpha = 0.01$ is 3.36. Based on the provided data, it can be inferred that the t-count score is lower than the t-table score when the concentration of added Cd²⁺ metal ions range from 0.1 to 0.20 ppm. The findings suggest that the threshold concentration of Cd²⁺ metal ions, below which there is no discernible impact on the growth of *Chaetoceros Calcitrans*, lies in the range between 0.1 and 0.20 ppm. Furthermore, the highest concentration of Cd²⁺ metal ions that the phytoplankton *Chaetoceros Calcitrans* can tolerate without adverse effects

is 0.20 ppm. This observation suggests that *Chaetoceros Calcitrans* exhibits significant tolerance against Cd²⁺ metal ion pollution.

Based on the established permissible threshold of 0.1 ppm for Cd²⁺ metal ions in water bodies, it may be inferred that *Chaetoceros Calcitrans* phytoplankton can sustain regular growth even in marine environments contaminated with Cd²⁺

metal ions. This finding demonstrates that *Chaetoceros Calcitrans* has the potential to serve as a bioindicator for marine environments infected with Cd²⁺ metal ions. Figure 3 displays the data pertaining to the percentage of growth inhibition (PGI) of *Chaetoceros Calcitrans* in relation to the concentration of Cd²⁺ metal ions.

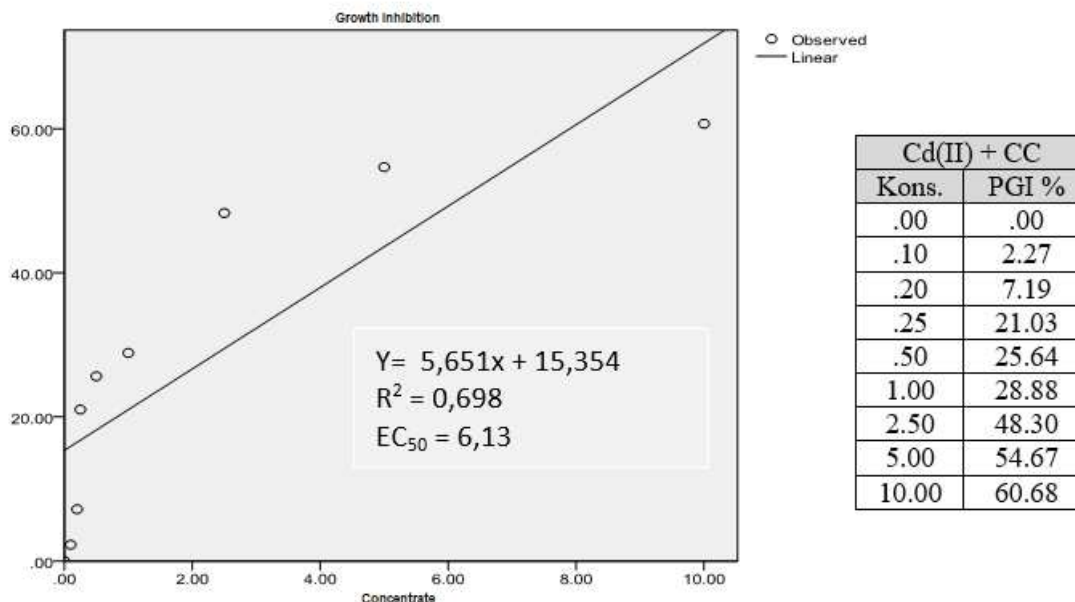


Fig. 3 Relationship between Cd²⁺ metal ion concentration and percentage of growth inhibition (PGI) in *Chaetoceros Calcitrans*

The determination of the EC50 price involves plotting the PGI price against the concentration of Cd²⁺ metal ions injected, as illustrated in Figure 3. The regression line equation can be expressed as $y = 5.651x + 15.514$. The EC₅₀ price was determined to be 6.13 ppm. The observed ability of *Chaetoceros Calcitrans* to withstand elevated levels of Cd²⁺ metal ions suggests that phytoplankton may contribute to the detoxification of Cd²⁺ metal ions. The process of detoxifying Cd²⁺ metal ions by phytoplankton encompasses a minimum of two sequential steps:

- The activation of *Phytochelatase synthase* (PC synthase) is initiated by elevated intracellular levels of Cd²⁺ metal ions, with glutathione (GSH) serving as the substrate.
- Compression and inactivation of Cd²⁺ metal ions for inclusion into the cytosol by *Phytochelatins* molecules.

Upon the entry of Cd²⁺ metal ions into the cytosol, an intricate mechanism associated with sulfur metabolism is triggered, leading to the synthesis of *Phytochelatins* (PC). When a *cysteine thiolic* group binds to Cd²⁺ metal ions, PC forms complexes with these ions (referred to as PC-Cd), thereby inhibiting the movement of free Cd²⁺ metal ions within the cytosol. The toxicity of the PC-Cd combination towards numerous plant enzymes is reduced by a factor of 1000 compared to the free metal ion Cd²⁺ [71]. Within minutes after the Cd²⁺ metal ions supply the enzyme, the process of self-regulation occurs, and PC synthesis continues until the Cd²⁺ metal ions are unavailable [72].

The process of PC synthesis has a high rate of speed, resulting in the formation of a complex with low molecular weight (LMW) in the presence of Cd²⁺ metal ions. The

presence of Cd²⁺ metal ions result in a complex with a greater medium molecular weight (MMW). These two complexes acquire S²⁻ ions within tonoplasts, forming high molecular weight (HMW) complexes that exhibit a greater connection for Cd²⁺ metal ions. This process facilitates the detoxification of Cd²⁺ metal ions. Due to the acidic pH of vacuoles, the HMW complex undergoes decomposition, resulting in the formation of novel complexes, including vacuolar organic acids like citric, oxalic, malic, and amino. Hydrolases in vacuoles can reproduce *Phytochelatins* in reverse reactions to the cytosol [73], [74].

IV. CONCLUSION

Based on the aforementioned research findings and treatment outcomes, we conclude that the utilization of Conway medium for the cultivation of marine phytoplankton *Chaetoceros Calcitrans*, at an initial density of 250,000 cells/mL of medium, yields a substantial increase in density, approximately 29-fold, within a mere six-day cultivation period. Furthermore, the introduction of Cd²⁺ metal ions at a concentration of 0.5 ppm into phytoplankton culture media of *Chaetoceros Calcitrans* has been observed to result in a reduction in the inhibited growth rate, as well as a drop in both the cell count and dry weight of *Chaetoceros Calcitrans*.

NOMENCLATURE

| | | |
|-------|------------------------|---------|
| N_t | cell density at time t | cell/mL |
| N_o | initial cell density | cell/mL |
| μ | inhibited growth rate | |

t the time (day).
 PGI percentage of growth inhibition.
 μ_i First inhibited growth rate setting
 μ_0 Control- inhibited growth rate settings.

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